



PATENT  
Docket No.: 19603/3355 (CRF D-1595E)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

|                |                                    |   |           |
|----------------|------------------------------------|---|-----------|
| Applicant(s) : | Barany et al.                      | ) | Examiner: |
|                |                                    | ) | Unknown   |
| Serial No. :   | 09/963,698                         | ) |           |
|                |                                    | ) |           |
| Cnfrm. No. :   | 2018                               | ) | Art Unit: |
|                |                                    | ) | 1627      |
|                |                                    | ) |           |
| Filed :        | September 26, 2001                 | ) |           |
|                |                                    | ) |           |
| For :          | DETECTION OF NUCLEIC ACID SEQUENCE | ) |           |
|                | DIFFERENCES USING THE LIGASE       | ) |           |
|                | DETECTION REACTION WITH            | ) |           |
|                | ADDRESSABLE ARRAYS                 | ) |           |

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SUPPLEMENTAL PRELIMINARY AMENDMENT

Commissioner for Patents  
Washington, D.C. 20231  
**Box: Non-Fee Amendment**

Dear Sir:

Please amend the above-identified patent application as follows:

In the Specification:

Please substitute the pending paragraphs at page 9, lines 30-31, page 9, lines 32-33, page 43, line 30 to page 44, line 10, and page 45, line 21 to page 46, line 2 with amended paragraphs at page 9, lines 30-31, page 9, lines 32-33, page 43, line 30 to page 44, line 10, and page 45, line 21 to page 46, line 2 as follows:

At page 9, lines 30-31:

Figures 21A-F show a schematic cross-sectional view of the synthesis of an addressable array, in accordance with Figures 19B-C.

At page 9, lines 32-33:

Figures 22A-C are schematic views of an apparatus used to synthesize the 8 x 8 array of 24 mers on a solid support in accordance with Figures 19B-C, 20A-C, and 21A-F.

At page 43, line 30 to page 44, line 10:

The starting surfaces will contain free amino groups, a non-cleavable amide linkage will connect the C-terminus of PNA to the support, and orthogonal side-chain deprotection must be carried out upon completion of segment condensation assembly in a way that PNA chains are retained at their addresses. A simple masking device has been designed that contains 200µm spaces and 200µm barriers, to allow each of 5 tetramers to couple to the solid support in distinct rows (Figure 20A). After addition of the first set of tetramers, the masking device is rotated 90°, and a second set of 5 tetramers are added (Figure 20B). This can be compared to putting icing on a cake as rows, followed by icing as columns. The intersections between the rows and columns will contain more icing, likewise, each intersection will contain an octamer of unique sequence. Repeating this procedure for a total of 6 cycles generates 25 squares containing unique 24-mers, and the remaining squares containing common 12-mers (Figures 20C and 21A-F). The silicon or glass surface will contain 10µm ridges to assure a tight seal, and chambers will be filled under vacuum. A circular manifold (Figure 26) will allow for circular permutation of the six tetramers prior to delivery into the five rows (or columns). This design generates unique 24-mers which always differ from each other by at least 3 tetramers, even though some sequences contain the same 3 tetramers in a contiguous sequence. This masking device is conceptually similar to the masking technique disclosed in Southern, et al., Genomics, 13:1008-1017 (1992) and Maskos, et al., Nucleic Acids Res., 21:2267-2268 (1993), which are hereby incorporated by reference, with the exception that the array is built with tetramers as opposed to monomers.

At page 45, line 21 to page 46, line 2:

Figures 21A-F show a schematic cross-sectional view of the synthesis of an addressable array (legend). Figure 21A shows attachment of a flexible spacer (linker) to surface of array. Figure 21B shows the synthesis of the first rows of oligonucleotide tetramers. Only the first row, containing tetramer 1, is visible. A multi-chamber device is placed so that additional rows, each containing a different tetramer, are behind the first row. Figure 21C shows the synthesis of the first columns of oligonucleotide tetramers. The multi-chamber device or surface has been rotated 90°. Tetramers 9, 18, 7, and 12 were added in adjacent chambers. Figure 21D shows the second round synthesis of the oligonucleotide rows. The first row contains tetramer 2. Figure 21E shows the second round of synthesis of oligonucleotides. Tetramers 34, 11, 14, and 23 are added in adjacent chambers during the second round. Figure 21F shows the structure of the array after third round synthesis of columns (the first row contains tetramer 3), adding tetramers 16, 7, 20, 29. Note that all 24-mer oligonucleotides within a given row or column are unique, hence achieving the desired addressable array. Since each 24-mer differs from its neighbor by three tetramers, and tetramers differ from each other by at least 2 bases, then each 24-mer differs from the next by

at least 6 bases. Each mismatch significantly lowers  $T_m$ , and the presence of 6 mismatches in just 24 bases would make cross hybridization unlikely even at 35°C. Note that the smaller 12-mer sequences are identical with one another, but are not at all common with the 24-mer sequences. Even though the particular 12-mer sequence may be found within a 24-mer elsewhere on the grid, for example 17-1-2-3-28-5, an oligonucleotide will not hybridize to the 12-mer at temperatures above 50°C.

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**REMARKS**

Pursuant to 37 CFR § 1.121, attached as Appendix A is a Version With Markings to Show Changes Made.

In view of the all of the foregoing, applicants submit that this case is in condition for allowance and such allowance is earnestly solicited.

Respectfully submitted,

Date: December 30, 2001

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I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Commissioner for Patents, Washington, D.C. 20231, on the date below.

12/20/01  
Date

Peggy Birmyer  
Peggy Birmyer

## Appendix A

### Version With Markings to Show Changes Made

In reference to the amendments made herein to the specification, additions appear as double-underlined text, while deletions appear as bracketed text, as indicated below:

#### In the Specification:

At page 9, lines 30-31:

Figures [21A-G] 21A-F show a schematic cross-sectional view of the synthesis of an addressable array, in accordance with Figures 19B-C.

At page 9, lines 32-33:

Figures 22A-C are schematic views of an apparatus used to synthesize the 8 x 8 array of 24 mers on a solid support in accordance with Figures 19B-C, 20A-C, and [21A-G] 21A-F.

At page 43, line 30 to page 44, line 10:

The starting surfaces will contain free amino groups, a non-cleavable amide linkage will connect the C-terminus of PNA to the support, and orthogonal side-chain deprotection must be carried out upon completion of segment condensation assembly in a way that PNA chains are retained at their addresses. A simple masking device has been designed that contains 200µm spaces and 200µm barriers, to allow each of 5 tetramers to couple to the solid support in distinct rows (Figure 20A). After addition of the first set of tetramers, the masking device is rotated 90°, and a second set of 5 tetramers are added (Figure 20B). This can be compared to putting icing on a cake as rows, followed by icing as columns. The intersections between the rows and columns will contain more icing, likewise, each intersection will contain an octamer of unique sequence. Repeating this procedure for a total of 6 cycles generates 25 squares containing unique 24-mers, and the remaining squares containing common 12-mers (Figures 20C and [21A-G] 21A-F). The silicon or glass surface will contain 10µm ridges to assure a tight seal, and chambers will be filled under vacuum. A circular manifold (Figure 26) will allow for circular permutation of the six tetramers prior to delivery into the five rows (or columns). This design generates unique 24-mers which always differ from each other by at least 3 tetramers, even though some sequences contain the same 3 tetramers in a contiguous sequence. This masking device is conceptually similar to the

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Figures [21A-G] 21A-F show a schematic cross-sectional view of the synthesis of an addressable array (legend). Figure 21A shows attachment of a flexible spacer (linker) to surface of array. Figure 21B shows the synthesis of the first rows of oligonucleotide tetramers. Only the first row, containing tetramer 1, is visible. A multi-chamber device is placed so that additional rows, each containing a different tetramer, are behind the first row. Figure 21C shows the synthesis of the first columns of oligonucleotide tetramers. The multi-chamber device or surface has been rotated 90°. Tetramers 9, 18, 7, and 12 were added in adjacent chambers. Figure 21D shows the second round synthesis of the oligonucleotide rows. The first row contains tetramer 2. Figure 21E shows the second round of synthesis of oligonucleotides. Tetramers 34, 11, 14, and 23 are added in adjacent chambers during the second round. Figure 21F shows the structure of the array after third round synthesis of [PNA rows. The first row contains tetramer 3. Figure 21G shows the structure of the array after third round synthesis of] columns[,] (the first row contains tetramer 3), adding tetramers 16, 7, 20, 29. Note that all 24-mer oligonucleotides within a given row or column are unique, hence achieving the desired addressable array. Since each 24-mer differs from its neighbor by three tetramers, and tetramers differ from each other by at least 2 bases, then each 24-mer differs from the next by at least 6 bases. Each mismatch significantly lowers  $T_m$ , and the presence of 6 mismatches in just 24 bases would make cross hybridization unlikely even at 35°C. Note that the smaller 12-mer sequences are identical with one another, but are not at all common with the 24-mer sequences. Even though the particular 12-mer sequence may be found within a 24-mer elsewhere on the grid, for example 17-1-2-3-28-5, an oligonucleotide will not hybridize to the 12-mer at temperatures above 50°C.